PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Goldman et al.	Examiner: Q. Nguyen #9
Serial No.	:	09/846,588	
Cnfrm. No.	:	To Be Assigned	Art Unit: 1636
Filed	:	May 1, 2001	
For	:	METHOD OF INDUCING NEURONAL PRODUCTION IN THE BRAIN AND SPINAL CORD	

DECLARATION OF STEVEN A. GOLDMAN UNDER 37 C.F.R. §1.132

U.S. Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

I, STEVEN A. GOLDMAN, pursuant to 37 C.F.R. § 1.132, declare:

1. I received B.A. degrees in Biology and Psychology from the University of Pennsylvania in 1978, a Ph.D. degree in Neurobiology from Rockefeller University in 1983, and an M.D. degree from Cornell University Medical College in 1984.

2. I am a Professor of Neurology and Neuroscience at Weill Medical College of Cornell University, as well as the Nathan Cummings Professor of Neurology at Cornell, and an Attending Neurologist at New York Presbyterian Hospital.

3. I am a named inventor of the above patent application.

4. As described in my above patent application, my laboratory has found that viral overexpression of brain-derived neurotrophic factor ("BDNF") in the normal adult mammalian ventricular system induces the generation of new neurons from the progenitor cell population of the ventricular subependyma. The new neurons migrate to the olfactory bulb primarily, but a large cohort invades the neostriatum as well, wherein they integrate as new neurons. These cells adopt a DARPP32/ GABAergic/calbindin$^+$ phenotype,

Considered by Q.N. 3/6/03

characteristic of the medium spiny neuronal population of the caudate-putamen. Since this is the predominant neostriatal phenotype lost in Huntington's Disease, I postulated that the induced generation of this cell type could slow or revere disease progression.

5. My students and I tested this strategy for treatment of Huntington's Disease in a transgenic model of Huntington's Disease known as the R6/2 mouse. This mouse has been engineered to express a 150 repeat polyglutamine expansion in the first exon of the ht gene (Mangiarini et al. Cell 87:493-506 (1996)). The mutant mouse develops increasing spasticity beginning by 6-8 weeks of age, exhibits striatal degeneration and intranuclear inclusions, and typically dies by 12-14 weeks of age. These traits make it an attractive target for cell-based therapeutic strategies, in that its baseline deterioration is both rapid and reproducible, providing a clear time frame within which therapeutic strategies may be best evaluated.

6. To test the feasibility of using an adenovirus containing a BDNF coding sequence ("AdBDNF") to induce striatal neurogenesis to treat Huntington's Disease, students in my laboratory working under my direction injected AdBDNF intraventricularly into Huntington mutant R6/2 mice and into normal wild-type mice. The results are described below.

7. The huntingtin mutant R6/2 mouse brain continued to harbor competent neuronal progenitor cells which can give rise to striatal neurons in response to AdBDNF infection of the ventricular zone. We determined that >140 new medium spiny neurons/mm^3/2-3 weeks, or by extrapolation 2,400-3,640/mm^3/year, may be induced in response to AdBDNF.

8. The normal wild-type mouse neostriatum harbors approximately 40,000 neurons/mm^3. Against this baseline, huntingtin mutant mice of the same strain and genetic background lose >10% of their striatal neurons by 8 weeks of age, and >15% by 12 weeks (quantification of these figures in the 12 week transgenic mouse is ongoing; these mice typically die by 12-14 weeks). Our data suggest that with an appropriately long-lasting expression vector, a majority of the lost striatal neuronal population may be regenerated in response to BDNF, on an annualized basis. Moreover, I anticipate that when used in conjunction with strategies intended to expand the underlying neural progenitor population, BDNF overexpression can induce regeneration of medium spiny neurons in the huntingtin brain more rapidly than they are lost to the underlying disease process.

9. By labeling the newly generated neostriatal neurons, by injections of a retrograde tracer into the globus pallidus, which is the normal target of neostriatal medium spiny neurons, we found that >40% of the newly-generated striatal neurons extended fibers to their targets in the globus pallidus by 7 weeks. I would expect even higher proportions of neostriatal neurons to extend fibers and connect to their target neurons at longer time points.

10. The new neurons survived after their initial generation, with no significant fall in the numbers of newly generated neurons between 3 and 8 weeks after being generated.

11. On the basis of these observations, I believe there is a reasonable likelihood that the increment in new medium spiny cells regenerated in response to AdBDNF treatment will be sufficient to yield a deceleration of the disease course, as reflected in a diminished morbidity and/or mortality. Proof of this contention in humans will need to await clinical trials of BDNF overexpression vectors in Huntington's Disease patients. That being said, I am optimistic that this strategy will indeed prove beneficial in either slowing the disease course, or otherwise beneficially affecting the clinical status of these patients.

12. In addition, I believe that this demonstration of the inducibility of endogenous neural progenitor cells provides us both a conceptual and operational basis for using neurotrophins besides BDNF, as well as gene delivery of such other neurotrophins, to stimulate endogenous progenitor cells of the adult brain, for the purpose of regenerating neural cell populations lost to disease or injury. An example in support of this contention has recently been published by Nakatomi et al. (Cell 110: 429-441, 2002), which reported that FGF may be used to induce the production of new hippocampal pyramidal neurons.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 12-17-02

Steven A. Goldman, M.D., Ph.D.